October 1, 2019

MRIGlobal
% Fran White
President
MDC Associates, LLC
180 Cabot Street
Beverly, Massachusetts 01915

Re: K183462
Trade/Device Name: Applied Biosystems *Bacillus anthracis* Detection Kit
Regulation Number: 21 CFR 866.4000
Regulation Name: Device to detect and identify biothreat microbial agents in human clinical specimens
Regulatory Class: Class II
Product Code: QIF, OOI
Dated: December 13, 2018
Received: December 14, 2018

Dear Fran White:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's

requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kristian Roth, Ph.D.
Branch Chief
Bacterial Multiplex and Medical Countermeasures Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K183462

Device Name
Applied Biosystems(TM) Bacillus anthracis Detection Kit

Indications for Use *(Describe)*
The Applied Biosystems Bacillus anthracis Detection Kit is a real-time polymerase chain reaction (PCR) test kit intended for the qualitative in vitro diagnostic (IVD) detection of target DNA sequences for Bacillus anthracis (B. anthracis, or BA). The Applied Biosystems Bacillus anthracis Detection Kit is intended to test human whole blood (EDTA) specimens and blood culture specimens with growth detected by a continuous monitoring blood culture system. Blood culture specimens must be determined to contain gram-positive bacilli by Gram stain prior to testing. Testing of whole blood specimens must be performed concomitantly with standard of care blood culture.

The Applied Biosystems Bacillus anthracis Detection Kit is indicated for use in CLIA-certified high-complexity laboratories in response to a confirmed Bacillus anthracis event only in accordance with the guidelines provided by public health authorities prior to or during a public health emergency. Testing with the Applied Biosystems Bacillus anthracis Detection Kit must only be performed when public health authorities have determined the need for this test. The test must only be used with specimens from individuals with clinical signs and symptoms of B. anthracis infection and who have either been exposed to B. anthracis or may have been exposed to B. anthracis.

The Applied Biosystems Bacillus anthracis Detection Kit is intended for use as an aid in the diagnosis of anthrax infection and results are for the presumptive identification of Bacillus anthracis. The diagnosis of B. anthracis infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence, in addition to the identification of B. anthracis from cultures or directly from clinical specimens. The definitive identification of B. anthracis requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be required.

The Applied Biosystems Bacillus anthracis Detection Kit has not been clinically evaluated with specimens collected from individuals with B. anthracis infection or those presumed to be exposed to B. anthracis. 'B. anthracis Not detected' results do not preclude infection with Bacillus anthracis and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Laboratories implementing this test must have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing B. anthracis. Anthrax is a nationally notifiable disease caused by a biothreat microbial agent and must be reported to public health authorities.

The distribution of in vitro diagnostic devices for Bacillus spp. detection is limited to laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities.

The Applied Biosystems Bacillus anthracis Detection Kit is intended for use with the ABI 7500 Fast Dx Real-Time PCR Instrument with analysis using the Applied Biosystems Bacillus anthracis Interpretive Software (BaIS).

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

Date of Summary: **September 27, 2019**

Product Name Applied Biosystems™ *Bacillus anthracis* Detection Kit

Sponsor
MRIGlobal
425 Volker Boulevard
Kansas City, Missouri 64110

Correspondent
MDC Associates, Inc.
Fran White, President
180 Cabot Street
Beverly, MA 01915
Phone: (978) 705 5011
Fax: (866) 540 3448 Email: regulatory@mdcassoc.com

Common Name
Device to detect and identify biothreat microbial agents in human clinical specimens

Product Classification
866.4000

Classification
QIF, Class II

Substantial Equivalency

Characteristic	**MRIGlobal** Applied Biosystems™ *Bacillus anthracis* Detection Kit *(New Device)*	**BioFire Defense, Inc.** FilmArray NGDS Warrior Panel K170883 *(Predicate Device)*
Similarities		
Product Code	QIF	PRD
Intended Use	The Applied Biosystems *Bacillus anthracis* Detection Kit is a real-time polymerase chain reaction (PCR) test kit intended for the qualitative *in vitro* diagnostic (IVD) detection of target DNA sequences for *Bacillus anthracis* (*B. anthracis*, or BA). The Applied Biosystems *Bacillus anthracis* Detection Kit is intended to test human whole blood (EDTA) specimens and blood culture specimens with growth detected by a continuous monitoring blood culture system. Blood culture specimens must be determined to contain gram-positive bacilli	The FilmArray® NGDS Warrior Panel is a qualitative, multiplexed, nucleic acid-based *in vitro* diagnostic test intended for use with the FilmArray® 2.0 system. The FilmArray® NGDS Warrior Panel detects and identifies *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus nucleic acids directly from human whole blood (EDTA). The FilmArray® NGDS Warrior Panel is also intended to be used to test for

Characteristic	**MRIGlobal** Applied Biosystems™ *Bacillus anthracis* Detection Kit *(New Device)*	**BioFire Defense, Inc.** FilmArray NGDS Warrior Panel K170883 *(Predicate Device)*
	by Gram stain prior to testing. Testing of whole blood specimens must be performed concomitantly with standard of care blood culture. The Applied Biosystems *Bacillus anthracis* Detection Kit is indicated for use in CLIA-certified high-complexity laboratories in response to a confirmed *Bacillus anthracis* event only in accordance with the guidelines provided by public health authorities prior to or during a public health emergency. Testing with the Applied Biosystems *Bacillus anthracis* Detection Kit must only be performed when public health authorities have determined the need for this test. The test must only be used with specimens from individuals with clinical signs and symptoms of *B. anthracis* infection and who have either been exposed to *B. anthracis* or may have been exposed to *B anthracis*. The Applied Biosystems *Bacillus anthracis* Detection Kit is intended for use as an aid in the diagnosis of anthrax infection and results are for the presumptive identification of *Bacillus anthracis*. The diagnosis of *B. anthracis* infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence, in addition to the identification of *B. anthracis* from cultures or directly from clinical specimens. The definitive identification of *B. anthracis* requires additional testing and confirmation procedures in consultation with the appropriate public health authorities for whom reports may be required. The Applied Biosystems *Bacillus anthracis* Detection Kit has not been clinically evaluated with specimens collected from individuals with *B. anthracis* infection or those presumed to be exposed to *B. anthracis*. '*Bacillus anthracis* not detected' results do not preclude infection with *Bacillus anthracis* and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.	*Bacillus anthracis or Yersinia pestis* nucleic acids in blood cultures that are determined to be positive either by an automated system, by turbidity, or by daily Gram stain. In addition, the FilmArray® NGDS Warrior Panel may also be used to detect and identify *Yersinia pestis and Francisella tularensis* nucleic acids directly from sputum specimens. The FilmArray® NGDS Warrior Panel is intended to test individuals with signs and symptoms of infection from biothreat agents and/or individuals who are at risk for exposure or may have been exposed to these agents. The FilmArray® NGDS Warrior Panel is indicated as an aid in the diagnosis of anthrax, plague, tularemia, Q fever, and the hemorrhagic fevers caused by Ebola and Marburg viruses, in response to a suspected or confirmed bioterrorism event or outbreaks. It is for diagnostic use in conjunction with other clinical, epidemiologic, and laboratory data, in accordance with the guidelines provided by the appropriate Department of Defense and public health authorities. Results are for the presumptive identification of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus. The definitive identification of *Bacillus anthracis*, *Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus requires additional testing and confirmation procedures in consultation with the appropriate Department of Defense and public health authorities for whom reports may be necessary.

Characteristic	**MRIGlobal** Applied Biosystems™ *Bacillus anthracis* Detection Kit *(New Device)*	**BioFire Defense, Inc.** FilmArray NGDS Warrior Panel K170883 *(Predicate Device)*
	Laboratories implementing this test must have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing *B. anthracis*. Anthrax is a nationally notifiable disease caused by a biothreat microbial agent and must be reported to public health authorities. The distribution of *in vitro* diagnostic devices for *Bacillus* spp. detection is limited to laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities. The Applied Biosystems *Bacillus anthracis* Detection Kit is intended for use with the ABI 7500 Fast Dx Real-Time PCR Instrument with analysis using the Applied Biosystems *Bacillus anthracis* Interpretive Software (BaIS).	
Methodology/ Technological Principle	Real-time-PCR	Nested PCR with melt curve analysis
Specimen types	Whole Blood or Blood Culture	Whole Blood; Blood Culture (for *Bacillus anthracis and Yersinia pestis*), Sputum (for *Yersinia pestis* and *Francisella tularensis*)
Assay Targets	DNA sequences unique to *B. anthracis*	Nucleic acid sequences unique to *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus
Result Output	Qualitative detection of *B. anthracis* DNA	Qualitative detection of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella burnetii*, Ebola virus, and Marburg virus nucleic acids
Test Interpretation	Automated test, interpretation, and report generation	Automated test, interpretation, and report generation
Differences		
Multiplex Capability	Multiplexed assay. Two gene targets and internal process control run in single reaction.	Multiplexed assay for detection of *Bacillus anthracis, Yersinia pestis, Francisella tularensis, Coxiella*

Characteristic	**MRIGlobal** Applied Biosystems™ *Bacillus anthracis* Detection Kit *(New Device)*	**BioFire Defense, Inc.** FilmArray NGDS Warrior Panel K170883 *(Predicate Device)*
		burnetii, Ebola virus, and Marburg virus in a single analysis run.
Sample Extraction	Qiagen QIAamp™ DNA Mini Blood Kit (manual) and Roche MagNA Pure Automated Nucleic acid Extraction (automated)	Automated in FilmArray pouch
PCR Reagents	Lyophilized reagents are formulated as a complete Mastermix and are reconstituted upon sample addition with no requirement to add water or buffer.	Reagents contained within FilmArray pouch
Instrumentation	Applied BioSystems® 7500 Fast Dx [K082562]	FilmArray 2.0
Throughput	96-well format allows for simultaneous analysis of up to 93 patient samples (+ controls) for detection of two *Bacillus anthracis* targets and an internal process control on a single plate	One sample at a time.
User Complexity End User	High Complexity Targeted CLIA-certified, sentinel laboratories (commercial diagnostic laboratories, hospital-based laboratories and clinical institutions) in the event of a public health emergency	Low/Moderate Complexity The FilmArray® NGDS Warrior Panel is solely for use by United States Department of Defense laboratories, and laboratories designated by the Department of Defense.

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Intended Use

The Applied Biosystems *Bacillus anthracis* Detection Kit is a real-time polymerase chain reaction (PCR) test kit intended for the qualitative *in vitro* diagnostic (IVD) detection of target DNA sequences for *Bacillus anthracis* (*B. anthracis*, or BA). The Applied Biosystems *Bacillus anthracis* Detection Kit is intended to test human whole blood (EDTA) specimens and blood culture specimens with growth detected by a continuous monitoring blood culture system. Blood culture specimens must be determined to contain gram-positive bacilli by Gram stain prior to testing. Testing of whole blood specimens must be performed concomitantly with standard of care blood culture.

The Applied Biosystems *Bacillus anthracis* Detection Kit is indicated for use in CLIA-certified high-complexity laboratories in response to a confirmed *Bacillus anthracis* event only in accordance with the guidelines provided by public health authorities prior to or during a public health emergency. Testing with the Applied Biosystems *Bacillus anthracis* Detection Kit must only be performed when public health authorities have determined the need for this test. The test must only be used with specimens from individuals with clinical signs and symptoms of *B. anthracis* infection and who have either been exposed to *B. anthracis* or may have been exposed to *B anthracis*.

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The Applied Biosystems *Bacillus anthracis* Detection Kit has not been clinically evaluated with specimens collected from individuals with *B. anthracis* infection or those presumed to be exposed to *B. anthracis*. '*Bacillus anthracis* not detected' results do not preclude infection with *Bacillus anthracis* and should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.

Laboratories implementing this test must have the appropriate biosafety equipment, personal protective equipment (PPE), containment facilities and personnel trained in the safe handling of diagnostic clinical specimens potentially containing *B. anthracis*. Anthrax is a nationally notifiable disease caused by a biothreat microbial agent and must be reported to public health authorities.

The distribution of *in vitro* diagnostic devices for *Bacillus* spp. detection is limited to laboratories that follow public health guidelines that address appropriate biosafety conditions, interpretation of test results, and coordination of findings with public health authorities.

The Applied Biosystems *Bacillus anthracis* Detection Kit is intended for use with the ABI 7500 Fast Dx Real-Time PCR Instrument with analysis using the Applied Biosystems *Bacillus anthracis* Interpretive Software (BaIS).

Methodology

The Applied Biosystems™ *Bacillus anthracis* Detection Kit is a multiplexed real-time polymerase chain reaction (PCR) test kit intended for the qualitative *in vitro* diagnostic (IVD) detection of target DNA sequences for *B. anthracis*. Reagents are lyophilized in a 96-well plate format as a fully formulated Mastermix and are stable at room temperature for up to one year. The kit is specifically designed for performing real-time PCR using the Applied Biosystems (ABI) 7500 Fast Dx instrument and software, with nucleic acids extracted from clinical specimens using a Qiagen manual extraction method or Roche MagNA Pure automated extraction methods. An automated interpretative software component (BaIS) is included in the kit but supplied separately and operates on a computer(s) that is separate from the ABI 7500 Fast Dx computer.

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Performance Data

Analytical Sensitivity

Limit of Detection (expressed as CFU/mL) was determined using *Bacillus anthracis* Ames, tested with twenty technical replicates per reagent lot of the Applied Biosystems™ *Bacillus anthracis* Detection Kit to achieve >95% detection when averaged across three reagent lots. Limit of Detection (LOD) was independently confirmed for whole blood, aerobic blood culture, and anaerobic blood culture for each extraction method, Qiagen DSP DNA Blood Mini Kit and Roche MagNA Pure using the Roche MagNA Pure LC 2.0 Robot. One thousand six hundred and twelve technical replicates were tested to determine LOD. See Table 1 below.

Table 1. Limit of Detection (expressed as CFU/mL) determined for Whole Blood, Blood Culture, Aerobic and Anaerobic per Extraction Method

Extraction Method	Matrix	Limit of Detection (CFU/mL)
Qiagen DSP DNA Blood Mini Kit	Whole Blood	150
	Blood Culture, Aerobic	10,000
	Blood Culture, Anaerobic	10,000
Roche MagNA Pure	Whole Blood	50
	Blood Culture, Aerobic	2270
	Blood Culture, Anaerobic	3040

Analytical Inclusivity

Quantified stocks of 24 different *Bacillus anthracis* strains representing geographic, temporal, genotypic, and phenotypic diversity of the species were prepared in culture under ATCC-recommended conditions and quantitated by enumeration on agar media to evaluate inclusivity using the Applied Biosystems™ *Bacillus anthracis* Detection Kit. Each BA inclusivity panel member was spiked into whole blood (K2 EDTA), at 3X the established LoD (expressed as CFU/mL) and processed using both extraction methods in triplicate – Roche MagNa Pure (MNP) extraction robot and Qiagen DSP DNA Blood Mini Kit per the kit instructions. The detection rate for both extraction methods was 100% for *B. anthracis* strains that carry both plasmid targets. For three strains that are known to carry only one plasmid target, the assay generated '*Bacillus anthracis* suspected' results as expected.

Inclusivity *in silico* Analysis Summary

All available *Bacillus anthracis* genome assemblies were downloaded from NCBI and imported into the CLC Genomics Workbench (Qiagen) for a total of 144 panel members. A local BLAST within the software was then performed with imported assemblies against the amplicon sequence for each assay. A list of assemblies without amplicon hits was generated for each assay to further investigate assemblies not producing hits. The results indicated that assemblies that did not produce a hit contained only chromosome sequences or only the alternate plasmid. Several assemblies showed very low coverage of plasmids, likely due to absence of the plasmid but presence of conserved regions in the genome, which have homology to small regions of plasmid sequences.

Analytical Exclusivity

Specificity of the Applied Biosystems™ *Bacillus anthracis* Detection Kit was evaluated by testing a panel of 154 non-target organisms which included near-neighbors and other bacteria, virus and fungi that might present with similar clinical presentation to those patients suspected of anthrax. For instances where bacteria, virus or fungi were not available as isolates, purified nucleic acid was used. Some known *Bacillus cereus* strains (BAG1X1-1 NR28575, 03BB102, and G-9241 NR-9564) contain a pXO1-like plasmid. Detection of these strains was indicated by a test result of '*Bacillus anthracis* suspected' with amplification of the plasmid manually confirmed by the supervisor. Initial no detection rate for exclusivity testing was 95.68%. Repeat testing was conducted for three organisms. Final no detection rate for exclusivity testing was 100%.

In addition, *in silico* exclusivity analysis was conducted with assay primers and probe positions aligned against additional non-target organisms. For *in silico* analysis, the results were filtered for: Exclusion of *Bacillus anthracis* hits; ≥80% for % Identity; and ≥80% for high-scoring segment pair (HSP) length (of the sequence for that primer or probe BLAST query).

Exclusivity *in silico* Analysis Summary

In silico exclusivity analysis was conducted with each of the assay primers and probes to identify non-target organisms with the potential to cross react with the assays. A BLAST search was performed against the NCBI nr, wgs, and genome databases. Organisms identified with high and reduced risk of amplification by *in silico* analysis were then evaluated by wet laboratory testing. Additionally, a specific set of organisms were screened by *in silico* analysis. A BLAST search of the primer and probe sequences was conducted. A primer and probe list was created and a BLAST search against each of the three databases performed for "all organisms" within the CLC Genomics Workbench software. For each primer or probe BLAST, the results were filtered for: (1) exclusion of *Bacillus anthracis* hits; (2) ≥80% for % identity; and (3) ≥80% for HSP length (of the sequence for that primer or probe BLAST query). The results of each BLAST were transferred to a Microsoft Excel spreadsheet and reviewed to identify organisms with "hits" for all three primer and probe sequences for each assay.

For the pXO1 assay, one organism (*Bacillus cereus* m1550) was identified as a match with 100% identity to the primer and probe sequences as high potential to support amplification and was evaluated by wet laboratory testing. For the pXO2 assay, two organisms (*B. cereus* AH1272 and AH1273) were identified as matches with 100% identity to the probe sequence. However, through a secondary NCBI Primer BLAST, each strain also contains three SNPs in both primer binding sequences, reducing the likelihood to support amplification.

Interfering Substances

The Applied Biosystems™ *Bacillus anthracis* Detection Kit's potential to detect *Bacillus anthracis* in the presence of potentially interfering substances was evaluated. Fifty potentially interfering substances consisting of endogenous, exogenous, and technique-specific substances were tested for potential interference with the assay. Each substance was tested in whole blood with triplicate-paired samples in the presence and absence of *B. anthracis* Ames, spiked at 3X LoD. Endogenous substances were spiked at concentrations typically found in human whole blood or at the highest achievable concentration.

Technique-specific substances were spiked directly into PCR reactions at 2.5%, 5% and 10% v/v test concentrations.

The following technique-specific substances were found to interfere with the assay and appropriate limitation was added to the product labeling.

MagNA Pure Wash Buffer I
MagNA Pure Lysis/Binding Buffer
MagNA Pure Proteinase K
10% Bleach
Fisherbrand™ DNase Displace
QIAGEN Protease
QIAGEN Buffer AL
QIAGEN Buffer AW1 (with and without EtOH)
EtOH at greater than 5%

Microbial Interference

Detection of *Bacillus anthracis* in the presence of other clinically-relevant organisms, e.g. pathogens or normal flora that may be present in whole blood or positive blood culture specimens, was tested with the Applied Biosystems™ *Bacillus anthracis* Detection Kit. The detection rate of *B. anthracis* was 100% in the presence of potentially interfering organisms in whole blood and blood culture.

Reproducibility

Seven panel members were tested twice a day by three teams of two different operators on five non-consecutive days using two Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instruments across three different reagent lots to determine the reproducibility rate of detecting *Bacillus*

anthracis Ames with the Applied Biosystems™ *Bacillus anthracis* Detection Kit. Two teams performed extraction using the Qiagen QIAamp DSP DNA Blood Mini Kit and one team performed extraction using the Roche MagNA Pure LC. A breakdown of the concentrations tested is below:

HP = High Positive: organism spiked at 10 × LOD; 1.5e3 CFU/mL for DSP and 5e2 CFU/mL for MNP

LP = Low Positive: organism spiked at 3 × LOD; 4.5e2 CFU/mL for DSP and 1.5e2 CFU/mL for MNP

NEG = Negative: non-Ba organism spiked at 3 × Ba LOD; 4.5e2 CFU/mL for DSP and 1.5e2 CFU/mL for MNP

One low positive sample extracted by one operator returned negative results when tested against three reagent lots indicating an error during the sample preparation procedure. In a separate instance a high positive sample returned '*Bacillus anthracis* suspected' results when the pXO1 assay failed to amplify. The PCR for this sample was repeated and returned '*Bacillus anthracis* detected' results as expected. No false positive events occurred out of the 270 PCR tests of negative samples.
There were a total of 24 sample replicates (across all three reagent lots) that returned initial indeterminate results. All were determined to be negative by supervisor review. There were no invalid results for this study.

Carry-Over/Cross-contamination
The Applied Biosystems™ *Bacillus anthracis* Detection Kit workflow was evaluated for the potential for cross-contamination or sample carry-over during extraction using the automated system (Roche MagNA Pure) and the manual DSP (Qiagen) methods. For the automated method nine full MagNA Pure runs were performed with each run of 31 samples placed in a checkerboard fashion alternating high positive, spiked whole blood (K2 EDTA), blood culture aerobic and blood culture anaerobic and negative samples throughout the sample cartridge. Blood culture spiked samples were spiked with *Bacillus anthracis* Ames at 1 x 10^6 CFU/mL. Whole blood spiked samples were spiked with *Bacillus anthracis* Ames at 500 CFU/mL (10X LOD). For the manual method 12 batches of 15-24 samples each were performed using the DSP method with samples batched such that high positive samples were placed next to negative whole blood, aerobic blood culture and anaerobic blood culture. Blood culture spiked samples were spiked with *Bacillus anthracis* Ames at 1 x 10^6 CFU/mL. Whole blood spiked samples were spiked with *Bacillus anthracis* Ames at 1500 CFU/mL (10X LOD).

Two hundred and seventy-nine samples were extracted on the MagNA Pure in nine separate runs and resulting extracts were analyzed in singlet PCR reactions. A total of two false positive events occurred out of a total of 135 negative samples analyzed, resulting in a specificity rate of 98.5%.

Two hundred and seventy-nine samples were manually extracted using the Qiagen DSP blood mini kit in twelve separate extraction batches and resulting extracts were analyzed in singlet PCR

reactions. No false positive events occurred out of a total of 139 negative samples analyzed, resulting in a specificity rate of 100%.

Test area surface monitoring control swabs were collected at various steps in the workflow during execution of the Carryover/Cross-Contamination study to support a detailed evaluation of sample handling and processing steps that have the potential to introduce contamination.

One hundred and sixty-two test area surface monitoring control swabs were collected during nine separate MagNA Pure runs for the Carryover/Cross-Contamination study. Swabs were processed and analyzed in singlet PCR. Results were used to determine areas of the instrument and steps in the process that are at high risk of cross-contamination. A total of nine swab locations were tested to include three swab locations in the BSC and six surfaces inside the MagNA Pure work deck. Contamination events occurred on one BSC surface location and five surfaces of the MagNA Pure during testing.

Two hundred and sixteen test area surface monitoring control swabs were collected during twelve separate DSP extraction batches for the Carryover/Cross-Contamination study. Swabs were processed and analyzed in singlet PCR. Results were used to determine areas of the BSC and steps in the process that are at high risk of cross-contamination. One location (BSC - left surface - after loading DSP columns) was identified as having a higher rate of contamination during the workflow.

Clinical Specificity

To demonstrate clinical specificity, the Applied Biosystems™ *Bacillus anthracis* Detection Test System was tested using left-over fresh and frozen blood culture samples, (confirmed by Gram stain to be positive for any bacterial species and selectively positive for Gram Positive rods), and blood culture samples spiked with any bacterial species and a subset spiked with Gram positive rod bacteria. Randomly accessed, residual blood – blood collected in K2EDTA (e.g., CBC, tested in hematology) and with no correlation to positive blood culture specimens, for which standard of care testing was performed (patients may or may not have fever, but specimens were obtained for other laboratory testing) was collected from Hematology laboratories for immediate testing with the Applied Biosystems™ *Bacillus anthracis* Detection Kit. All samples (blood culture and whole blood) were collected prospectively and serially without further selection criteria. Additionally, febrile whole blood samples were collected from consented patients presenting with fever and flu-like symptoms. Samples were collected from three (3) point-of-care collection sites including physician offices, urgent care centers or hospital clinics/ER. Clinical performance testing was conducted at three laboratories within the US. Febrile whole blood samples were shipped to one laboratory on the day of collection and were processed within 24 hours of receipt.

No reference method testing was performed.

All samples were assumed to be negative for *Bacillus anthracis*.

The study generated '*Bacillus anthracis* not detected' results for the 401 blood culture specimens and 439 whole blood specimens included in the performance analyses, demonstrating 100% negative percent agreement when compared to the expected negative result.

Clinical Sensitivity

To demonstrate product sensitivity, the Applied Biosystems™ *Bacillus anthracis* Detection Test System was tested using whole blood specimens collected from febrile patients presenting with fever of unknown origin and flu-like symptoms. Following testing of febrile whole blood specimens determined to be negative for *Bacillus anthracis*, simulated samples positive for *Bacillus anthracis* were contrived using unique febrile whole blood specimens each spiked with *Bacillus anthracis* at or near LOD. Simulated positive samples were prepared and tested in BSL-3 laboratory facilities. Each febrile whole blood aliquot was spiked with 1 of 18 strains of *Bacillus anthracis*. Contrived samples were randomized with negative, unspiked whole blood samples and blinded to the operators. Two different operators processed samples using either the Qiagen DSP manual method or with the Roche MagNA Pure automated robot. Two different instruments were used to analyze samples. A minimum of 25 samples were spiked with *Bacillus anthracis* Ames at LOD and additional samples were spiked at 3× LOD with 1 of 17 other *Bacillus anthracis* strains.

The study generated '*Bacillus anthracis* detected' results for 84 of 87 low-positive whole blood specimens. Three specimens initially generated '*Bacillus anthracis* suspected' results due to detection only one plasmid target. Final positive percent agreement was 96% (CI: 90.4-96,5%).